

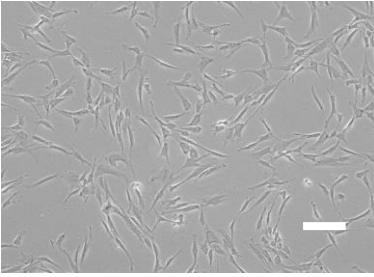
Supplementary Figures 1-5

Ocular instillation of conditioned medium from mesenchymal stem cells is effective for dry eye syndrome by improving corneal barrier function

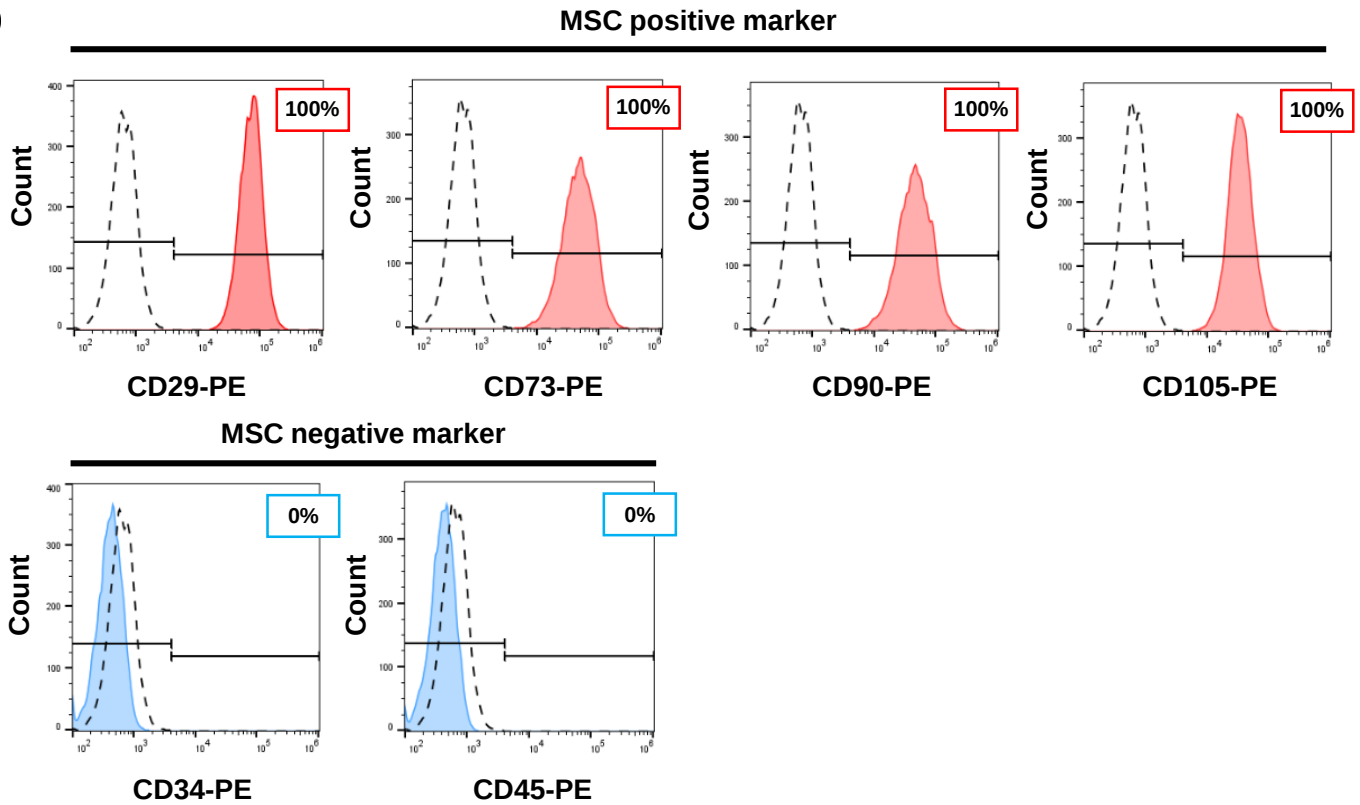
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Supplementary Figure S1

a

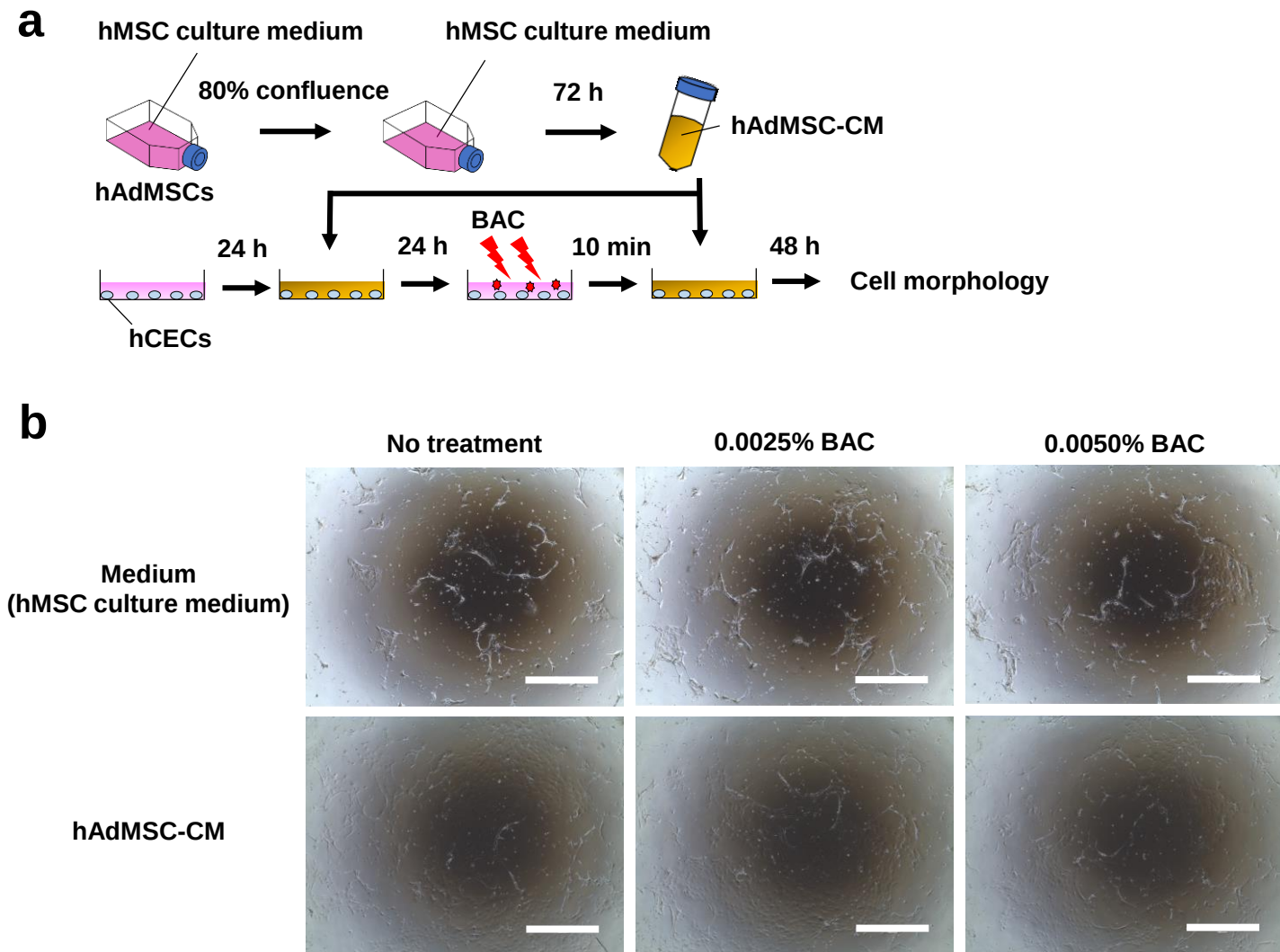


b



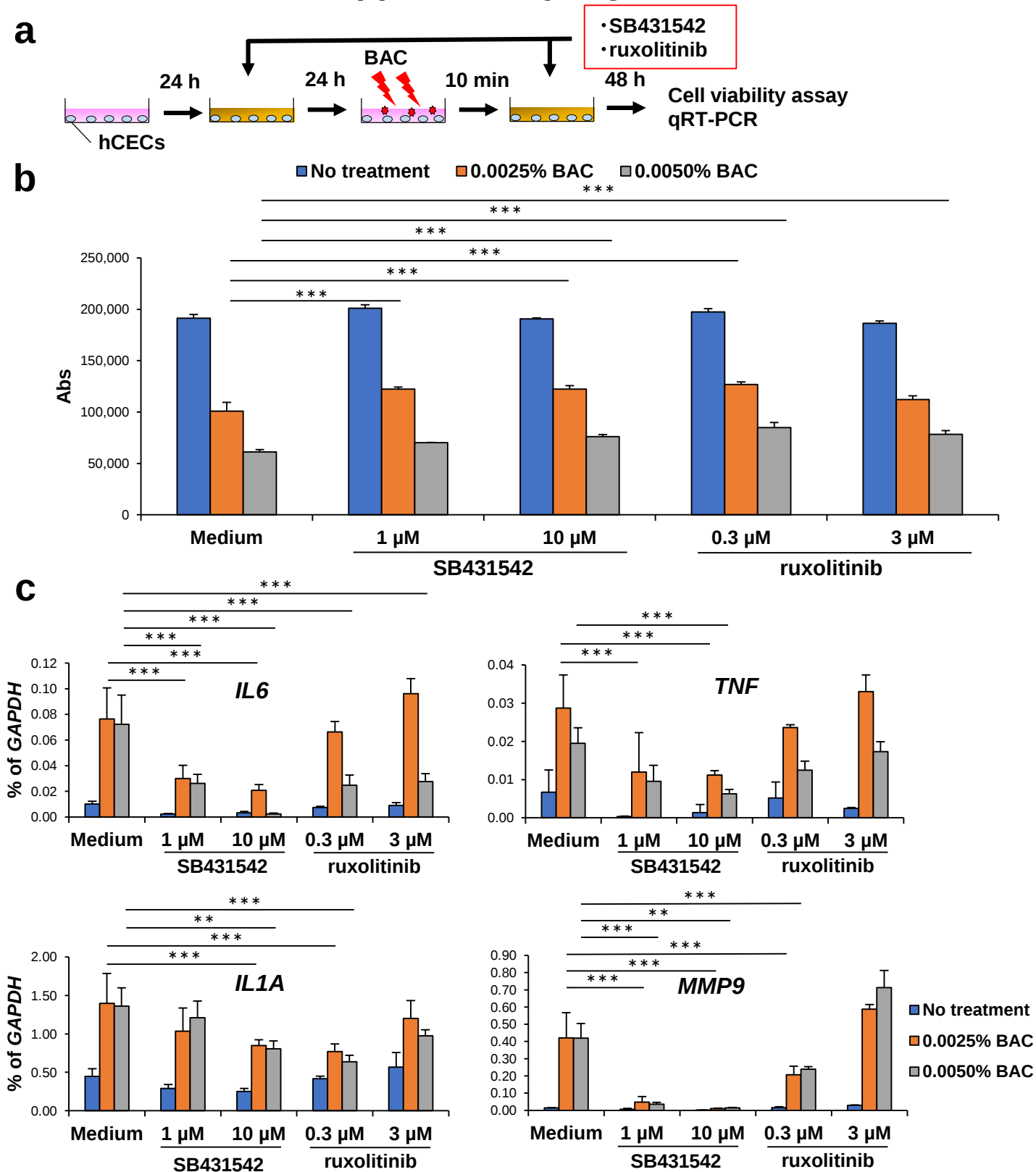
Supplementary Figure S1. Characteristics of hAdMSCs. (a) Phase images of hAdMSCs. Scale bar, 200 μ m. $n = 12$ biological replicates. (b) Expression of hMSC positive and negative markers. $n = 3$ biological replicates. hAdMSCs; human adipose-derived mesenchymal stem cells.

Supplementary Figure S2



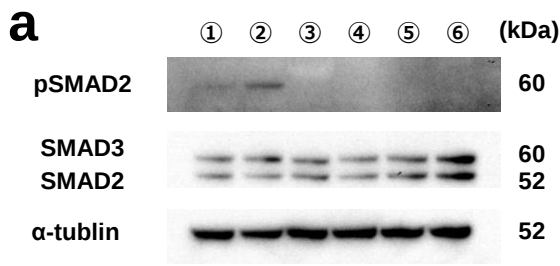
Supplementary Figure S2. Morphology of hCECs could not be maintained by hAdMSC-CM derived from hMSC culture medium. (a) Schematic representation of hAdMSC-CM preparation using hMSC culture medium and the evaluation of hAdMSC-CM on hCECs. (b) Phase images of hCECs 2 days after treatment with or without BAC. Scale bar, 200 μm . $n = 9$ biological replicates. hAdMSC-CM; conditioned medium of human adipose-derived mesenchymal stem cells, BAC; benzalkonium chloride, hCECs; human corneal epithelial cells.

Supplementary Figure S3

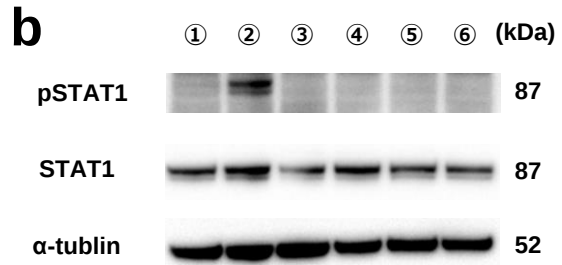


Supplementary Figure S3. BAC-induced cytotoxicity and inflammation of hCECs are suppressed by inhibition of TGF β and JAK-STAT signalling pathways. (a) Schematic representation of the evaluation of SB431542 and ruxolitinib on BAC-induced cytotoxicity and inflammation of hCECs. (b) Cell viability assay of hCECs 2 days after treatment with or without BAC. The results are presented as the mean $\pm$ SD; $n = 4$ biological replicates. *** $p < 0.001$. (c) Expression levels of inflammation-related genes in hCECs 2 days after treatment with or without BAC. The results are presented as the mean $\pm$ SD; $n = 4$ biological replicates. ** $p < 0.01$, and *** $p < 0.001$.

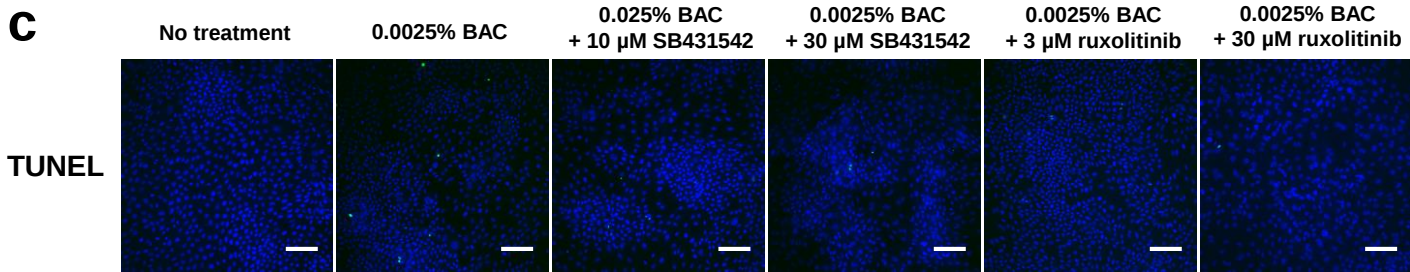
Supplementary Figure S4



- ① No treatment
- ② 0.0025% BAC
- ③ 10 μ M SB431542
- ④ 0.0025% BAC + 10 μ M SB431542
- ⑤ 30 μ M SB431542
- ⑥ 0.0025% BAC + 30 μ M SB431542



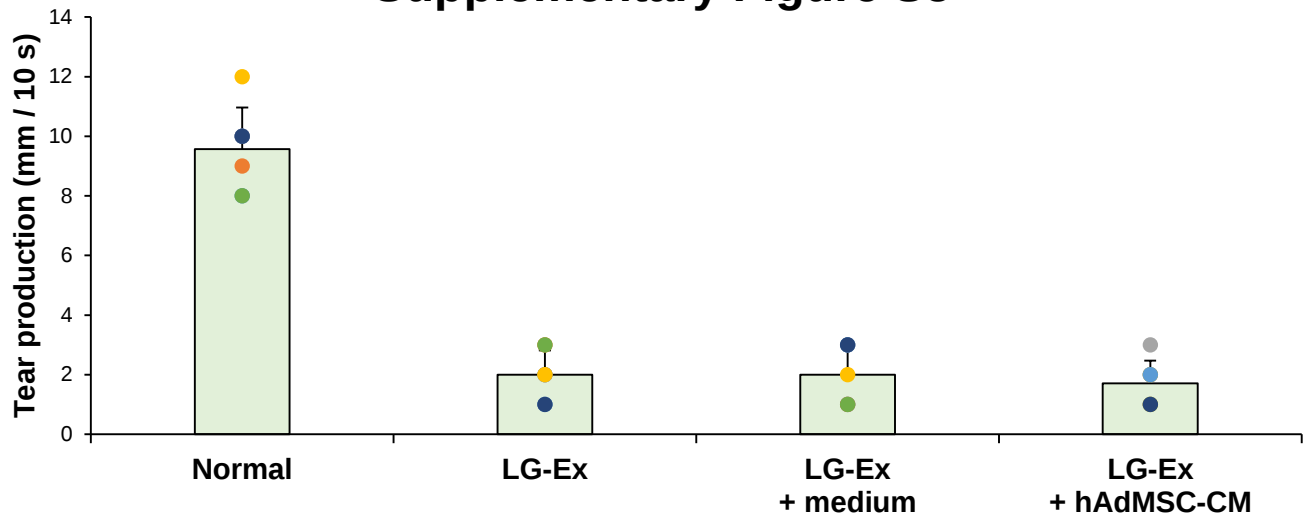
- ① No treatment
- ② 0.0025% BAC
- ③ 3 μ M ruxolitinib
- ④ 0.0025% BAC + 3 μ M ruxolitinib
- ⑤ 30 μ M ruxolitinib
- ⑥ 0.0025% BAC + 30 μ M ruxolitinib



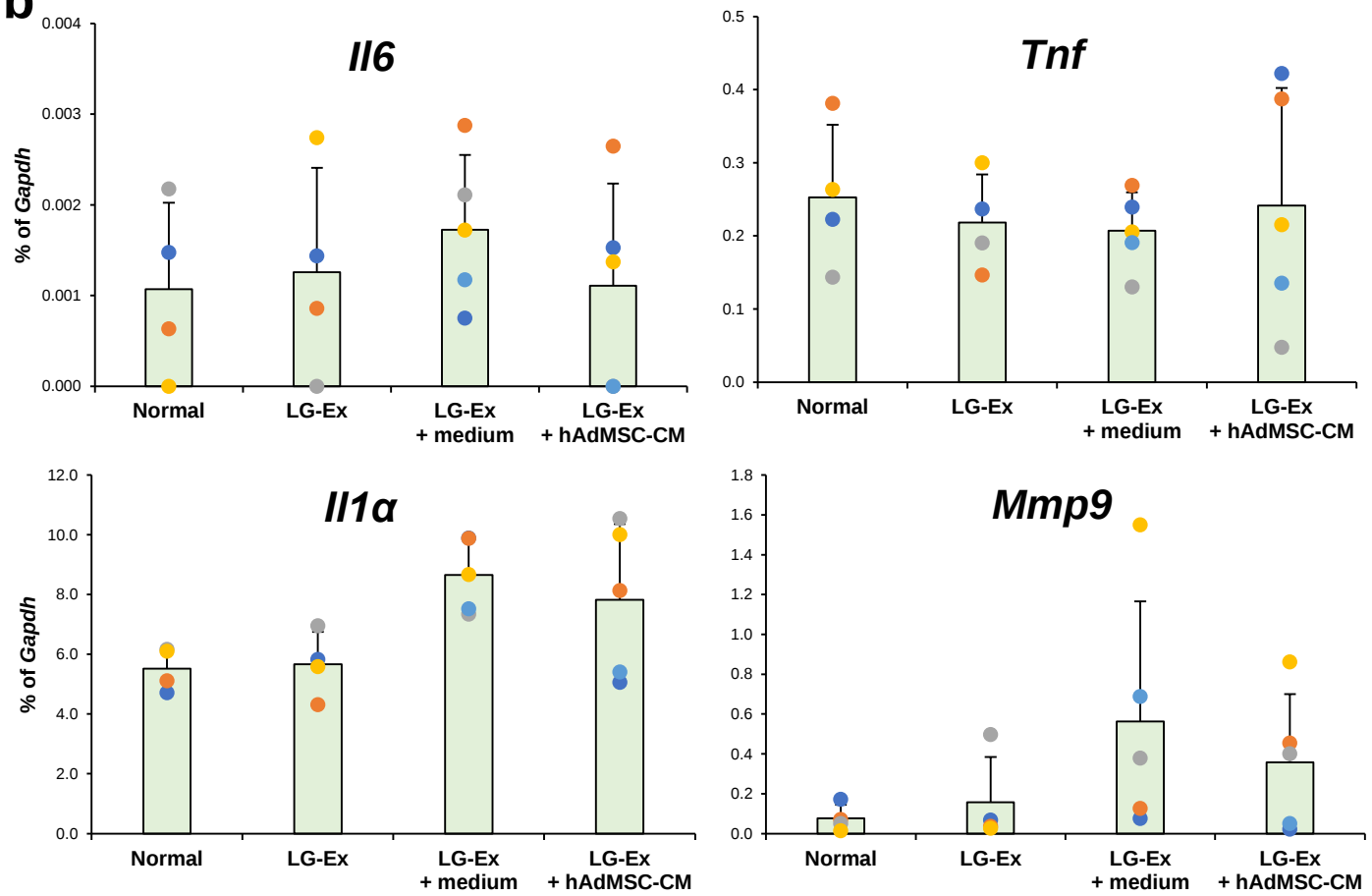
Supplementary Figure S4. Number of apoptotic cells was not increased by inhibition of TGF β and JAK-STAT signalling pathways. (a) Expression of TGF β signalling-related proteins. (b) Expression of JAK-STAT signalling-related proteins. (c) Fluorescein staining images of TUNEL in hCECs 1 day after treatment with or without BAC. Scale bar, 100 μ m. $n = 4$ biological replicates. TUNEL; Terminal deoxynucleotidyl transferase dUTP nick end labelling.

Supplementary Figure S5

a



b



Supplementary Figure S5. Examination of tear production and corneal inflammation in LG-Ex rats.

(a) Measurement of tear production in SD (Normal), LG-Ex, LG-Ex with ocular instillation of medium (LG-Ex + medium), and LG-Ex with ocular instillation of hAdMSC-CM (LG-Ex + hAdMSC-CM) rats 1 week after ocular instillation. The results are presented as the mean $\pm$ SD; $n = 7$ biological replicates. (b) Expression levels of inflammation related-genes in the cornea of Normal, LG-Ex, LG-Ex + medium, and LG-Ex + hAdMSC-CM rats. The results are presented as the mean $\pm$ SD; $n = 4$ (Normal and LG-Ex) and $n = 5$ (LG-Ex + medium and LG-Ex + hAdMSC-CM) biological replicates.